BIOL 4140
Fall 2020

Protocol

Cytotoxicity and Alamar Blue Viability Assay

The goal of this lab is to both become comfortable working with and interpreting the results of the Alamar Blue viability assay, but also to gain a further understanding of the complexities associated with establishing cytotoxicity.

Materials

Nonsterile

- 96-well plate with confluent HFF cells (provided)
- D10
- 2ml of Alamar Blue 10x (150mg/L)
- waste container for tips
- media waste container

Procedure

1. Working at the bench, remove the media or drug treatment from wells using a multichannel or single channel pipettor. NOTE: recall that you do not want to leave cells exposed without media for any longer than necessary.
2. Add 100ul of fresh D10 media to each well.
3. Add 20uL of Alamar Blue solution to each well.
4. Incubate in the 37°C CO_2 incubator for 1-4 hours.
5. Read the fluorescence levels using the plate reader: excitation 530-560nm, emission: 590nm.
6. If time allots, look at your cells under phase-contrast.
7. Dispose of tips in sharps. Add BDD to media waste and then rinse waste media down the drain.
8. Incubate overnight and read again using the plate reader.

*Note you may want to use a multichannel pipette, however, the error among the multiple tips may greatly affect your assay. Consider using a multichannel for removing liquids only and use a single tip pipette to add accurate volumes.